

The template binding region of the primer hybridises to template nucleic acid from a sample. The region is of any convenient design available to the person of ordinary skill and is limited only by practical considerations. It may be DNA, RNA or other provided that it provides a substrate for polymerase mediated primer extension. Template binding can be effected at any desired stringency, that is to say under appropriate hybridisation stringency conditions the template binding region of the primer may hybridise to the template region (if present in the template) to the exclusion of other regions. Alternatively template binding may be effected at reduced stringency to extend the primer on any convenient number of related template sequences, such as for example human leukocyte antigen (HLA) genes, or other conserved genes, particularly bacterial or ribosomal RNA genes. Primers may be provided wherein the template binding regions are members of a set of random hexamer sequences. Thus the expression "a complementary sequence" is intended to include all sequences outlined above provided that the template binding region and hybridisation conditions allow the desired degree of sequence discrimination. By way of example the template binding region may be 100%, up to 95%, up to 90%, up to 85%, up to 80%, up to 75%, or up to 70% complementary to the corresponding template sequence. The template binding region is conveniently of 6-50 nucleotides such as 10-40 nucleotides, 15-30 nucleotides, particularly 20-30, 17-22, 16-23 or 15-24 nucleotides. Each of the above ranges is a separate and independent embodiment of the invention. All of the above applies in an analogous manner to the target binding region of the primer with the proviso that the target binding region is in general shorter than the template binding region, examples of convenient and preferred ranges are set out hereinafter. It will be appreciated that the overall selectivity of the method of the invention may be applied in an allele-specific or multiple allele manner for the template binding or target binding regions independently. Each permutation is a particular aspect of the invention.

As outlined above, the target binding region may if desired comprise a non-copiable species such as 2'-O-methyl RNA, peptide nucleic acid (PNA) and variants of these. In this case a separately identifiable linker is not required and the target binding region is considered to comprise a linker separating the template binding and target binding regions. The target binding region may be shorter than those traditionally designed for hybridisation to amplicons (amplification products) since the amplicon-target interactions of this invention are

unimolecular and hence kinetically (and thermodynamically) more favoured than bi-molecular interactions. By way of non-limiting example, the target binding region may comprise no more than 6, such as no more than 7, no more than 8 no more than 9 or no more than 10 nucleotides.

5 It will be understood that the tail of the primer may include additional nucleotides complementary to part of the template binding region in the primer. These may be used to "fine tune" the affinity of the primer tail for complementary sequences.

10 The linker separates the template binding and target binding regions. Optimum characteristics for the linker may be determined by routine experimentation. Whilst we do not wish to be bound by theoretical considerations, the linker may comprise no more than 200 nucleotides or less such as 100 or 50 nucleotides. In general these regions are kept close together, we believe this may favour hybridisation of target binding region to the target region. In a preferred aspect the linker comprises a non-amplifiable moiety such as HEG, alone or combined with further nucleotides, more preferably alone. Where the template binding region and the tail region of the primer are arranged to prevent polymerase-mediated copying of the primer tail the linker may be a direct bond.

20 In a further aspect of the invention we provide a nucleic acid primer comprising (i) a template binding region and (ii) a tail comprising a linker and a target binding region such that in use the target binding region hybridises to a complementary sequence in an extension product of the primer corresponding to the target nucleic acid. The template binding region and the tail region are preferably arranged such that the tail region remains single stranded in the PCR amplification products. More preferably a blocking moiety is sited between the template binding region of the primer and the tail region, which moiety prevents polymerase mediated chain copying of the tail region of the primer template. A particular blocking moiety is a hexethylene glycol (HEG) monomer. The target binding region is preferably selected to hybridise to a complementary target sequence in the primer extension product less than 200 such as less than 100 base pairs, such as less than 50 base pairs, such as less than 40 base pairs, less than 30 base pairs less than 25 or less than 20 base pairs such as less than 15, 10 or even 5 from a sequence complementary to the template binding region in the primer.

30 Hybridisation of the target binding region in the tail of the primer to a complementary sequence in the primer extension product corresponding to the target nucleic acid causes a

detectable change in the signalling system. Any convenient signalling system may be used, by way of non-limiting example we refer to the measurement of the change in fluorescence polarisation of a fluorescently labelled species (European Patent No. 0 382 433 - Zeneca Limited), DNA binding proteins, creation of restriction sites in duplex species for endpoint  
5 detection, the bringing together of elements to give a target site, the incorporation of detectably (modified) dNTPs into primer extension products and further probe species. In addition any convenient sequence specific species may be used, examples include intercalators such as wavelength specific intercalators, also species used to form triplex structures. Convenient intercalators will be apparent to the scientist skilled in the art (cf.  
10 Higuchi et al, BioTechnology, 1992, 10, 413-417).

Further systems include two-component systems where a signal is created or abolished when the two components are brought into close proximity with one another. Alternatively a signal is created or abolished when the two components are separated following binding of the target binding region.

Both elements of the two component system may be provided on the same or different molecules. By way of example the elements are placed on different molecules, target specific binding displaces one of the molecules into solution leading to a detectable signal.

Convenient two-component systems may be based on the use of energy transfer, for example between a fluorophore and a quencher. In a particular aspect of the invention the detection system comprises a fluorophore/quencher pair. Convenient and preferred attachment  
20 points for energy transfer partners may be determined by routine experimentation. A number of convenient fluorophore/quencher pairs are detailed in the literature (for example Glazer et al, Current Opinion in Biotechnology, 1997, 8, 94-102) and in catalogues such as those from Molecular Probes, Glen and Applied Biosystems (ABI). Any fluorescent molecule is suitable  
25 for signalling provided it may be detected on the instrumentation available. Most preferred are those compatible with the 488 nm laser of the ABI PRISM 7700 (Fluorescein and Rhodamine derivatives). The quencher must be able to quench the dye in question and this may be via a Fluorescence Resonance Energy Transfer (FRET) mechanism involving a second, receptor fluorophore, or more preferably via a collisional mechanism involving a non-fluorogenic  
30 quencher such as DABCYL, which is a "Universal" quencher of fluorescence. Furthermore it is preferred that the selected fluorophores and quenchers are easily incorporated into the